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Citation for published version:

Cordi, M, Ackermann, S, Bes, FW, Hartmann, F, Konrad, BN, Genzel, L, Pawlowski, M, Steiger, A, Schulz, H, Rasch, B & Dresler, M 2014, 'Lunar cycle effects on sleep and the file drawer problem', *Current biology : CB*, vol. 24, no. 12, pp. R549-50. <https://doi.org/10.1016/j.cub.2014.05.017>

Digital Object Identifier (DOI):

[10.1016/j.cub.2014.05.017](https://doi.org/10.1016/j.cub.2014.05.017)

Link:

[Link to publication record in Edinburgh Research Explorer](#)

Document Version:

Peer reviewed version

Published In:

Current biology : CB

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On lunar cycle effects on sleep and the file drawer problem

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Popular beliefs about full moon influence on humans exist, although no solid evidence has so far confirmed these ideas [1]. Cajochen et al. [2] now presented fascinating data on lunar cycle effects on human sleep EEG. However, in a re-analysis of sleep EEG data in three large samples, we were unable to replicate their findings. In addition, we identified further mostly unpublished null findings, suggesting that the conflicting results might be an example of a publication bias (i.e., the file drawer problem).

In a post hoc analysis of 64 sleep nights of 33 healthy volunteers, Cajochen et al. [2] found that nights recorded around the full moon were characterized by a 20-min reduction of total sleep time (TST), reduced time spent in sleep stage 4 (S4) as well as increased sleep latency (SL) and rapid eye movement (REM) sleep latency. Furthermore, delta activity during NonREM (NREM) sleep was 30% lower in nights around full moon compared to nights around new moon. Post hoc estimation of effects sizes revealed medium to large effects of lunar class on these sleep parameters (all $\eta^2 > .10$). As data were recorded in a light-controlled sleep laboratory setting, this study might be the first solid evidence confirming folk beliefs regarding lunar influences on objective measures of sleep.

However, the findings by Cajochen et al. [2] rely on a small number of participants and hence require replication. Here we re-analyzed three large data sets in search for lunar effects on sleep. Data set 1, recorded at the Max Planck Institute of Psychiatry, Munich, included 470 sleep EEGs from 366 healthy participants in a light-controlled sleep laboratory (see supplemental material for details). Lunar classes were defined as previously [2], with lunar class 1 representing full moon ± 4 nights, lunar class 2 covering the waxing and waning moon periods (full moon ± 5 -10 nights) and lunar class 3 representing new moon (full moon ± 10 -14 nights). In contrast to the results reported by Cajochen et al. [2], we did not find any evidence for an influence of lunar class on objective sleep parameters (all $P > .1$, see table 1).

Data set 2 was also recorded in the Max Planck Institute of Psychiatry, however in contrast to the first sample included 757 sleep EEGs of 29 volunteers each of whom slept for 13-60 consecutive

nights in the sleep laboratory, i.e. across all phases of the lunar cycle (see supplemental material).

Also in this data set we were not able to replicate any of the findings by Cajochen et al. [2] (see table 1).

Data set 3 represents a sample recruited at the University of Basel. It includes sleep EEGs of 870 young healthy volunteers who slept at home (see supplemental material). Again, there was no indication of a significant effect of the lunar cycle on sleep variables (see table 1). In addition, oscillatory activity during NREM sleep did not differ between lunar classes (see supplemental figure S1) in this data set. In particular, no effects were observed in the EEG delta band as reported by Cajochen et al. [2].

In sum, we were not able to replicate the large effects of lunar cycle on sleep EEG reported by Cajochen et al. [2] in three large samples consisting of 470, 757 and 870 sleep recordings: None of the variables that were reported to be affected by lunar cycle by Cajochen et al. yielded significant results in any of our samples. This also holds for alternative analyses with outlier exclusion and interaction analyses. Please note that for other sleep variables that were not significant in the data of Cajochen et al., some small and inconsistent effects and interactions with lunar class emerged (for details and discussion, see Supplemental Information).

Even though exact values of power calculations have to be interpreted with caution due to differences in research designs, in our three samples we can exclude the existence of similarly large lunar cycle effects as reported by Cajochen (e.g. effect sizes $\eta^2 > .10$) with high confidence: In the first data set, the statistical power to detect such effects was above 99%; in the second and third data set, we can exclude the existence of even smaller lunar cycle effects ($\eta^2 > .03$) on the respective sleep parameters with 99% confidence.

The present analysis suggests that the significant and consistent lunar cycle effects on sleep reported by Cajochen et al. [2] might be the result of other sources of variance. For example, as a post hoc re-analysis of an earlier study with a different focus, assignment of the subjects to lunar classes was not

randomized for age and sex. These two factors had significant main effects on most of those sleep variables that also showed significant lunar cycle effects. However, age and sex were unevenly (even though not significantly so) distributed across lunar classes in the Cajochen et al. [2] data set (e.g. almost three times as many older subjects in lunar class 1 compared to class 3 according to their table S3).

In addition, publications of re-analyses of data already ‘exploited’ otherwise are particularly prone for publication biases such as the “file drawer problem” [3]. Besides Cajochen et al. [2] and three authors of this correspondence (FWB, BR, MD), also several other researchers independently from each other had re-analyzed sleep data from earlier studies in search for lunar cycle effects: While the post hoc analysis of 1145 sleep diaries of 31 subjects reported similar results as Cajochen et al. [4], neither a re-analysis of 9778 sleep diary nights of 196 subjects [5] nor a re-analysis of a sleep diary study additionally using actigraphy to assess 5152 nights of 391 participants found any lunar cycle effects on sleep [6]. Also three prospective studies analyzing sleep diaries of cumulatively 6575 nights of 147 participants failed to find evidence for lunar cycle effects on sleep [7-9]. In sum, eight data sets with cumulatively 23600+ nights led to negative results, compared to two data sets with a total of 1209 nights reporting lunar cycle effects on sleep. Emphasizing the possibility of a file drawer problem, three of the studies with negative findings (among them the two largest samples) have never been published as full journal papers. However, we do not know how many more sleep researchers have already screened their sleep EEG data for lunar cycle influences without publishing their results. Thus, we would like to encourage others to report their findings. However, to overcome the obvious limitations of retrospective data analyses, carefully controlled studies specifically designed to address the question of lunar cycle influences on sleep using a within-subjects design in large samples are required for a definite answer.

Variable	Lunar Class 1		Lunar Class 2		Lunar Class 3		Sig. for Lunar Class	
	Mean	SE	Mean	SE	Mean	SE		
Munich1 (<i>n</i> = 470)	<i>n</i> = 153		<i>n</i> = 150		<i>n</i> = 167		<i>F</i> _{2, 465}	<i>P</i>
TST	415.9	±3.6	420.7	±3.5	420.7	±3.7	.42	.66
WASO	12.5	±1.0	11.5	±1.2	11.4	±1.1	.20	.82
SL2	21.3	±1.6	19.9	±1.5	19.7	±1.5	.61	.55
RL	108.5	±4.8	98.4	±3.2	103.0	±3.6	1.79	.17
Stage 1	9.7	±0.5	9.6	±0.4	9.4	±0.5	.06	.94
Stage 2	52.6	±0.8	53.3	±0.7	52.5	±0.6	.26	.77
Stage 3	9.5	±0.4	9.1	±0.3	10.0	±0.3	2.07	.13
Stage 4	7.6	±0.6	7.7	±0.6	8.0	±0.6	.01	.99
SWS	17.1	±0.7	16.7	±0.6	18.0	±0.7	.69	.50
NREM	69.7	±0.6	70.1	±0.5	70.5	±0.5	.31	.73
REM	20.6	±0.4	20.4	±0.4	20.1	±0.4	.27	.77
Munich2 (<i>n</i> = 757)	<i>n</i> = 238		<i>n</i> = 260		<i>n</i> = 259		<i>F</i> _{2, 745}	<i>P</i>
TST	440.0	±2.6	437.7	±2.4	436.7	±2.1	1.41	.24
WASO	2.1	±0.3	1.9	±0.2	2.6	±0.3	.89	.41
SL2	14.9	±1.0	15.2	±0.8	15.9	±0.8	.21	.81
RL	78.6	±2.5	75.8	±1.9	73.6	±1.8	.50	.61
Stage 1	6.6	±0.2	7.0	±0.2	7.5	±0.3	.02	.98
Stage 2	48.0	±0.4	48.2	±0.5	48.9	±0.5	.94	.39
Stage 3	10.9	±0.2	10.8	±0.2	10.7	±0.2	2.48	.08
Stage 4	10.9	±0.4	10.5	±0.5	9.6	±0.5	3.05	.05*
SWS	25.6	±0.5	24.6	±0.6	23.4	±0.6	2.37	.09
NREM	60.9	±1.0	61.4	±0.9	60.6	±0.9	2.04	.13
REM	21.9	±0.3	22.0	±0.3	21.9	±0.3	.35	.71
Basel (<i>n</i> = 870)	<i>n</i> = 260		<i>n</i> = 291		<i>n</i> = 319		<i>F</i> _{2, 863}	<i>P</i>
TST	450.1	±5.0	452.2	±4.3	453.7	±4.5	.05	.95
WASO	4.0	±0.5	3.6	±0.3	3.4	±0.3	.72	.49
SL2	20.5	±1.6	23.7	±2.1	20.5	±1.8	1.46	.23
RL	87.6	±2.6	88.5	±2.2	87.3	±2.6	.12	.89
Stage 1	3.4	±0.1	3.3	±0.1	3.3	±0.1	.56	.57
Stage 2	49.5	±0.5	49.5	±0.5	49.6	±0.4	.20	.82
Stage 3	11.3	±0.3	11.3	±0.3	11.3	±0.3	.02	.98
Stage 4	17.1	±0.4	16.3	±0.4	16.5	±0.4	.49	.61
SWS	28.5	±0.6	27.6	±0.5	27.8	±0.5	.31	.74
NREM	77.9	±0.4	77.1	±0.3	77.4	±0.3	1.45	.24
REM	18.7	±0.3	19.6	±0.3	19.3	±0.3	2.47	.09

Table 1. Sleep parameters for lunar classes 1 (full moon), 2 (waxing/waning moon) and 3 (new moon) for three different samples (Munich1, Munich2 and Basel). TST: total sleep time (in minutes); WASO: wake after sleep onset (in percent of TST); SL2: sleep latency to stage 2 (in min); RL: REM sleep latency (in min), Stage 1-4: sleep stages 1-4 (in percent of TST); SWS: slow wave sleep (sum of stages 3 and 4 in percent of TST); NREM: non-REM sleep (sum of stages 2, 3 and 4, in percent of TST), REM: REM sleep (in percent of TST). As indicated by an asterisk, sleep stage 4 in the Munich2 sample is the only variable yielding significance for lunar class ($P = .05$). Planned comparisons for this variable revealed a significant increase in stage 4 sleep during full moon (lunar class 1) as compared to new

moon (lunar class 3, $P = .04$), which is opposite to the result reported by Cajochen et al. None of the other planned comparisons for sleep parameters reported to be influenced by lunar cycle (TST, SL2, RL, stage 4, see [2]) were significant. For further analyses see supplemental material.

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Supplemental Information:

On lunar cycle effects on sleep and the file drawer problem

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Supplemental Procedures

Subjects

Data set Munich1: At the Max Planck Institute of Psychiatry, Munich, different studies [S1-S21 and in preparation] with cumulatively 470 nights from 366 subjects (164 female) were merged for a post hoc analysis on possible lunar influences on sleep. Subjects were aged 34.2 ± 15.3 years (range 18–74). All subjects were nonsmokers, did not take any medication (except contraceptives), and had no history of psychiatric or neurological disorders as assessed by interviews with psychiatrists. In addition, exclusion of sleep disorders was secured through screening of sleep EEGs by somnologists. 336 of the 366 subjects additionally underwent EEG, ECG and blood tests to screen for medical conditions before inclusion into the study. Menstrual cycle was documented/controlled for in 158 of the 164 female subjects, being either in the early follicular phase or postmenopausal. All studies were approved by ethics committees and all participants gave written informed consent prior to participation.

Data set Munich2: At the Max Planck Institute of Psychiatry, Munich, 757 nights were recorded from 29 healthy subjects (mean age 25.7 ± 5.2 , range 20-43; 10 females, 19 males). The main purpose of the long-term sleep recordings over series of nights was to acquire data on the course and stability of sleep parameters and REM-NREM cycles in normal subjects [S22]. Thus, the analysis of lunar influences on sleep was conducted post hoc. On average, 26 ± 10 (range 13-60) nights per subject were recorded in series of at least 13 consecutive nights, resulting in 238 female and 519 male nights. 25 subjects contributed nights from all three lunar classes, 4 subjects contributed nights from 2 lunar classes. All subjects were screened by a psychologist, had no sleep disturbances and reported to be free of any medication (except contraceptives).

Data set Basel: At the University of Basel, 870 healthy young subjects (mean age 22.6 ± 3.6 years, range 18-35; 588 females) participated in the study. Subjects participated in a study investigating the influence of genetic variations on sleep and memory [publication in preparation]. Thus, the analysis

of lunar influences on sleep was conducted post hoc. Participants were students or employees from the Basel area and were paid for their participation. Subjects did, by self-report, not take any medication (except contraceptives), and reported no neurological or mental illness. 300 female subjects took contraceptives, evenly distributed across lunar classes (51.4%/52.6%/49.3%). From all participants, 322 women provided data concerning the menstrual cycle, which was comparably distributed across lunar classes (36% / 39.4% / 47.2 % indicated being in the follicular phase; no significant difference in the distribution: $\chi^2 = 2.96$, $P = .23$). The study was approved by the local ethics committee and all participants gave written informed consent prior to participation.

Experimental procedure

Munich1: The data was collected between 1990 and 2013 during all seasons, with generally more nights collected in autumn and less in winter, independent of lunar class. Recordings were performed during all days of the week, with more recordings during weekdays compared to weekends during all three lunar classes. Subjects contributed a mean of $1.3 \pm .5$ nights (range 1-4); of 93 subjects with more than one night recorded, 48 subjects contributed nights out of at least two different lunar phases. All nights were recorded under identical light-controlled laboratory settings, i.e. halogen lights for EEG preparation and lights shut off from 23:00 hours on. All nights were preceded by adaptation nights in the sleep laboratory with the same schedule, however these nights were not included in the sample. Participants arrived in the sleep laboratory from 19:00-21:00 p.m. on, after which electrodes were placed. Participants were allowed to sleep from 23:00 (lights off) to 7:00 (lights on). All subjects were asked to maintain a regular sleep pattern comparable to the experimental and adaptation night during the week preceding the recordings and to refrain from excessive alcohol and caffeine consumption. On the testing day, they were asked to completely refrain from alcohol consumption and restrict their caffeine consumption to max. 1 cup and refrain from caffeine consumption from after noon on. All recordings stem from intervention-free baseline nights or placebo conditions at least one week apart from the verum condition in cases where the aim of the study included some kind of intervention (Mg²⁺, panamesin, progesterone, ghrelin, galanin, GHRH, GHRP). In lunar class 1, 153 nights were recorded (subjects' age range 19-73 years, mean age 35.5 ± 15.2 years, 70 females); in lunar class 2, 150 nights (age range 18-74 years, mean age 34.0 ± 15.6 years, 60 females); in lunar class 3, 167 nights (age range 18-74 years, mean age 33.3 ± 15.2 years, 80 females).

Munich2: The data was collected between 1972 and 1988 during all seasons, with generally more nights recorded in spring and less in winter, independent of lunar class. Due to the consecutive

recording over the course of several weeks in all subjects, all days of the week were comparably included. All recordings were performed in the same room of the sleep laboratory. During daytime, subjects followed their usual routine. In the evening, subjects arrived about one hour before bedtime for electrode hook-up and adaptation. Bedtime was adapted to the individual habitual bedtimes of the subjects, the average bedtimes and wake-up times did not differ between lunar classes (lunar class 1 bedtime 23:12, wake-up time 06:55; lunar class 2 bedtime 23:11, wake-up time 06:55; lunar class 3 bedtime 23:16, wake-up time 06:57; $P > .34$ and $P > .64$, respectively). In lunar class 1, 240 nights were recorded (subjects' age range 20-36 years, mean age 24.8 ± 3.8 years, 61 females); in lunar class 2, 315 nights (age range 20-43 years, mean age 25.5 ± 4.7 years, 103 females); in lunar class 3, 202 nights (age range 20-43 years, mean age 26.0 ± 4.1 years, 74 females).

Basel: The data of Basel used was collected between October 2008 and January 2011, during all seasons; more recordings were collected in autumn and less in winter, independent from lunar class. All recordings were collected on weekdays (on nights of Monday, Tuesday, Wednesday and Thursday). No data was collected on weekends. One single night of sleep was recorded, which subjects spent at home wearing a portable EEG recording device. To get used to wearing a portable EEG recording device, subjects spend a night at home wearing a portable dummy EEG recording device before entering the study. Participants' average bedtimes and wake-up times did not differ between lunar classes (lunar class 1 ($n = 254$) bedtime 00:12, wake-up time 08:20; lunar class 2 ($n = 285$) bedtime 00:07, wake-up time 08:17; lunar class 3 ($n = 312$) bedtime 00:08, wake-up time 08:20; $P > .70$ and $P > .93$, respectively). In lunar class 1, 260 nights were recorded (subjects' age range 18-35 years, mean age 22.4 ± 3.7 years, 175 females); in lunar class 2, 291 nights (age range 18-35 years, mean age 22.7 ± 3.7 years, 192 females); in lunar class 3, 319 nights (age range 18-35 years, mean age 22.7 ± 3.5 years, 221 females).

Sleep recordings

Munich1: Sleep data were recorded with a digital recorder (Comlab 32 Digital Sleep Lab, Brainlab Software, Schwarzer GmbH, Munich, Germany) from C3 and C4 electrodes and referenced against the mastoids. For standard polysomnography, electrooculogram, electrocardiogram and electromyogram were also recorded. The sampling rate was 250 Hz, the signal was filtered from .5 to 70 Hz.

Munich2: Sleep data were recorded with a 17-channel polygraph (Nihon-Kohden model EEG-4217) on paper (10mm/sec.) and stored on tape (14-channel Sabre VI, model 631). Recorded were 15

channels: 3 EEG (C3-A2, C4-A1, C3-C4 Ag-AgCl, mounted by collodium), 4 EOG (both eyes , horizontal and vertical electrode positions), 2 EMG (chin and submental), ECG, respiration (chest and air flow). Additional channels were for bed movements, sound and any floor vibrations. Time constant and filter settings for EEG, EMG and EOG according to standard requirements [S23]. All recordings were synchronized by a digital time signal (Johne & Reilhofer, ZG 16).

Basel: Sleep data in Basel were recorded at home using a mobile EEG recording device (Somnoscreen Neuro, Somnomedics, Germany). Six Ag-AgCl electrodes were placed according to the international 10–20 System (Fz, C3, Pz, Oz, left and right mastoid). Electrodes were physically referenced to Cz. Additionally, EOG, EMG and ECG were recorded for standard polysomnography. Finally, an actimeter was used to monitor movements. EEG signals were recorded between .2 – 35 Hz, EOG between .2 – 35 Hz, EMG and ECG between 1 – 128 Hz. The sampling rate for the EEG channels, as well as for the EMG and the ECG channels was 256 Hz, the sampling rate for the EOG channels was 128 Hz.

Habitual bed times in the Basel sample according to questionnaires were for subjects with EEG recordings in lunar class 1 (n = 258): 23:32; lunar class 2(n=290): 23:30; lunar class 3(n=318): 23:34. Habitual awakening times were for lunar class 1(n= 258): 07:53; lunar class 2(n= 290): 07:46; lunar class 3(n= 318): 07:50. Habitual sleep durations were for lunar class 1: 08h 21min; lunar class 2: 08h 16min; lunar class 3: 08h 16min. Actual bed times during the experimental nights were documented for 851 subjects of the 870 subjects included in the analyses. Mean time in bed \pm SEM were for lunar class 1 (n = 254): 08.20 \pm .09 hours; lunar class 2 (n = 285): 08.17 \pm .09 hours; lunar class 3 (n = 312): 08.20 \pm .09 hours. Time in bed did not differ across lunar classes, neither on the night when EEG was measured ($F(2, 848) = .08, P = .93$).

Sleep scoring

Munich1: Sleep data were visually scored for the 8 hours lights-off period by experts blind to the study aim according to standard criteria [S23].

Munich2: Sleep data were visually scored for the 8 hours lights-off period by two experts according to standard criteria [S23].

Basel: Analysis of sleep data in Basel was restricted to the period between the lights off and lights-on markers provided by the participants. If participants had forgotten these markers, sleep onset and offset were determined visually. For sleep stage analysis, data was referenced to the right mastoid. Sleep scoring of all sleep data was performed by an automatic algorithm (Somnolyzer 24x7) provided

by the Siesta Group according to the standard criteria [S23]. Scoring accuracy of the algorithm has been independently validated with an reported an agreement of 80% between automatic scoring procedures and a human expert scorer, while in comparison two visual human raters only approached an agreement of 77% [S24]. For the Basel data, the first 20 subjects were rescored manually after automatic scoring which also resulted in a match of more than 85%. Additionally to this promising amount of agreement, it was intended to use the same algorithm for all subjects of such a large sample size, recorded over several years, instead of having them scored by various different human scorers. For the total time in bed every 30-s epoch was scored as wakefulness, sleep stages 1, 2, 3, 4 and REM sleep, with slow wave sleep (SWS) defined by the sum of time spent in sleep stages 3 and 4. Sleep onset was defined by the first period in stage 1 sleep followed immediately by stage 2 sleep. REM sleep latency was determined with reference to sleep onset.

Frequency analysis

In addition to the classical sleep stage analysis, in the Basel sample an additional frequency analysis was performed. First, EEG data was re-referenced to the averaged mastoids. Data of Cz was re-instated during re-referencing. Then, sleep scoring data was imported and used as segmentation markers. Data was segmented to 30-second periods of wakefulness, stage 1 sleep, NonREM sleep (consisting of sleep stages 2, 3 and 4) and REM sleep. For frequency analysis, equally sized segments of EEG data consisting of 1024 data points (4 seconds) with 100 points overlap were created. Data quality was controlled by using an automatic artifact rejection procedure: Segments were kept for further analysis when (i) the maximal difference in EMG activity was $< 150\mu\text{V}$, (ii) the maximal voltage step in the each EEG channels (Fz, Cz, Pz, Oz) was $< 50\mu\text{V}/\text{ms}$ and (iii) the maximal difference in each channel was below $300\mu\text{V}$ ($500\mu\text{V}$ during NonREM sleep). On average $5.04\% \pm .36$ of a total number of 5611.98 ± 33.20 segments were excluded from analysis. Number of excluded segments did not differ between lunar classes ($P = .52$). Power in each frequency band was calculated in each artifact-free segment for each EEG channel using a Fast Fourier Transformation (FFT) with a 10% Hanning window (Resolution $.25\text{ Hz}$). Then, power spectra were averaged over all segments. Because 50Hz artifacts were greatest for Oz, this electrode was discarded from the analysis.

Statistical analyses

Data was analyzed according to Cajochen et al. with a mixed-model analysis of variance with between subjects factors “gender” (male, female) and “lunar class” (1, 2, 3) and the covariate “age”. Age was also used as a group factor in an additional analysis (see tables S3–S5). Where indicated by the ANOVA, follow-up t-tests for independent samples were conducted. In addition, we calculated planned pair-wise comparisons for the variables reported to be significant in the study by Cajochen et al. (see table 1). In the Basel sample, for each EEG derivation (Fz, Cz, Pz) and each EEG power density bin in the range between 0-25 Hz, an ANOVA with the above mentioned factors was calculated. A value of $P < .05$ was considered significant.

Alternative statistical analysis after outlier exclusion

For a more stringent view on the data, we also analyzed all data sets after exclusion of outliers. Outliers were defined as diverging by 3 or more standard deviations from mean in TST, S1, S2, SWS and REM. In the Munich1 sample, after exclusion of outliers, 414 nights of 312 subjects remained (whole sample: 472 nights), aged 34.5 years (± 15.2 , range 18-74), Lunar class 1: age 19 – 73 (35.5 ± 15.2), 83 males, 70 females, Lunar class 2: age 18 – 74 (33.9 ± 15.6), 91 males, 60 females, Lunar class 3: age 18 – 74 (33.2 ± 15.2), 88 males, 81 females. In the Munich2 sample, after exclusion of outliers 726 nights of 29 subjects remained (whole sample: 757 nights), aged 20 – 43, mean age 25.6 ± 4.5 . Lunar class 1: mean age 24.6 ± 3.7 , 176 male nights, 54 female nights; lunar class 2: mean age 25.5 ± 4.6 , 168 male nights, 81 female nights; lunar class 3: mean age 25.9 ± 5.0 , 157 male nights, 90 female nights. In the Basel sample, after exclusion of outliers 845 subjects contributing one sleep EEG each remained (whole sample: 870 nights), aged 22.6 years (± 3.6 , range 18-35 years); Lunar class 1: age 18 – 35 years (mean 22.3 ± 3.7 years), 82 males, 171 females; Lunar class 2: age 18 – 35 years (22.6 ± 3.6 years), 96 males, 188 females, Lunar class 3: age 18 – 35 years (22.7 ± 3.5 years) 92 males, 216 females. Results for these analyses are documented in supplemental table S2. In none of the three samples any of the findings of Cajochen et al. could be replicated with this analysis. Of note, both the Munich2 and Basel sample revealed a significant effect of lunar class on WASO, however in opposite direction.

Supplemental discussion and limitations

Variables: In none of the three data sets melatonin levels or subjective sleep quality measures were assessed. While for the latter several studies with large samples support our data (see main text), our failure to replicate lunar cycle effects on sleep measures cannot be generalized to the melatonin findings of Cajochen et al.

Sleep environment at home: In the Basel sample subjects slept at home, hence we could not control in as much they adhered to our inclusion criteria (no caffeine or alcohol; conducting quiet activities (e.g. as opposed to doing sports)). Furthermore, we could not control, in as much subjects pushed the marker button right before lying down to sleep (lights off), therefore sleep latency is difficult to interpret. We reanalyzed sleep latency for those who pressed the marker ($n = 825$): sleep latency did not differ between lunar classes ($P = .22$). Theoretically, uncontrolled conditions prevalent in the home-recorded study might have masked small lunar effects. However it must be noted that uncontrolled factors should have been randomly distributed across all lunar phases.

Automatic sleep scoring: Although we used a validated automatic sleep scoring algorithm for the Basel sample [S24], limitations of automatic sleep scoring need to be taken into account. The algorithm produces best results for correctly detecting wake, stage 2 sleep and REM sleep, with lower detection accuracy of stage 1, stage 3 and stage 4 sleep. Furthermore, sleep latency might be less accurate. However in our own validation of the automatic algorithm in the first 20 participants, we observed high agreement of the results of automatic scoring and visual sleep scoring by two independent raters ($> 85\%$). Furthermore, a possible bias of the automatic algorithm should be similar for all sleep record scorings and therefore without systematic effect on the three lunar classes. However, sensitivity of automatically scored data might be lower to detect any effects of lunar cycle on sleep.

Interventions: In the Munich1 sample, about half of the nights stem from intervention studies. In all studies, subjects were blind to the study condition. Only placebo/control nights were included in our sample, all of these were scheduled at least one week apart from the active treatment nights. It is theoretically possible that expectations about interventions in this sample could have influenced sleep, however these would have been randomly distributed across all lunar phases. In the Munich2 and Basel samples, studies did not include any interventions.

Contraceptives: In all three samples, the use of contraceptives was allowed, which might have had an effect on sleep. In the Munich1 and Munich2 samples, the use of contraceptives was not documented in all contributing studies. However, in the Munich1 sample, almost all female subjects

were either postmenopausal or in the early follicular phase of their menstrual cycle, hence they did not take contraceptives during the recordings. The Munich2 sample consists of long series of consecutive nights. In such a within-subject design, potential effects of contraceptive were thus equally distributed across lunar classes. In the Basel sample, about half of the female subjects took contraceptives, however these were evenly distributed across lunar classes: Lunar class 1: 51.4%, lunar class 2: 52.6%, lunar class 3: 49.3%.

Menstrual cycle: In the Munich2 sample, menstrual cycle was not documented. However, the sample consists of long series of consecutive nights, potential menstrual cycle effects were thus equally distributed across lunar classes. In the Basel sample, reported menstrual cycle was evenly distributed across lunar classes (follicular phase: 36.0%/39.4%/47.2%) with the three classes not differing in the distribution ($P = .23$).

Control of sleep times. While all recordings were preceded by an adaptation night and subjects were asked to follow a regular sleep schedule in the week preceding the recordings, none of the studies controlled the actual bedtimes during this time e.g. by actigraphy. At least in the Munich2 sample, this issue is of less a concern, since all subjects were recorded for many consecutive nights.

Caffeine consumption: In the Munich1 and Basel samples, subjects were not caffeine abstinent, but were asked to restrict their caffeine consumption to max. one cup per day and to abstain from caffeine after noon on the day of sleep recordings and also refrain from excessive caffeine consumption in the preceding week. Importantly, not only caffeine consumption, but also caffeine withdrawal in habitual drinkers has significant effects on wake EEG measures [S25] and sleep [S26]. Thus, caffeine consumption and caffeine abstinence both might act as confounders in sleep studies. While studying only caffeine-naïve subjects or continuously caffeine-abstinent subjects in sleep research would be ideal in this regard, availability of such subjects is restricted, rendering the inclusion of larger sample sizes difficult. However, studies with large sample sizes are less prone for potential caffeine consumption or withdrawal effects compared to smaller sample sizes, since such effects can be expected to be more evenly distributed across conditions, i.e. lunar classes in the present case. As an additional control measure for this issue, in the Basel sample we included high vs. low habitual caffeine consumption in a sub-sample ($n=250$) for which this information was documented as an additional control factor in the ANOVA. No significant effects of lunar class on sleep parameters resulted (all $P > .12$).

Variability of sleep data: Notable differences can be observed between the three data sets analyzed here and the data of Cajochen et al. concerning the variance of some variables. The most important influences for such variability are probably the following: 1. Sleep was recorded at home in the Basel

sample, where subjects were not awakened in the morning after a standardized 8 hour period as in the sleep laboratory, leading to larger variability in TST. 2. The Munich1 sample displayed the greatest variability in age, leading to larger variability in age-affected variables like WASO or S4. 3. Participants of the Munich2 sample slept for many consecutive nights in the sleep laboratory, resulting in optimal accommodation to the sleep lab environment, which in turn affected the variance of sleep variables, as e. g. WASO and S4. 4. The small sample studied by Cajochen et al. might have led to a sampling bias compared to the comparably larger three samples in our study. While an increased variability decreases statistical power, an increased number of observations, as in the three samples analyzed here, increases statistical power, thus compensating any potential effects due to differences in scatter of the data.

Methodological advances: Besides these caveats, our study implies a number of significant methodological improvements compared with the study of Cajochen et al. First, our results are based on very large sample sizes. Second, in contrast to the distribution in the Cajochen et al. study (cf. their supplemental table S3), in the three replication samples age was evenly distributed across lunar classes. Third, the data set Munich2 stems from a study conceived of as within-subject design, with every subject being recorded for at least 13 and up to 60 recurrent nights. This type of design is well suitable design to test lunar cycle effects, however, again the results do not support the assumption of a lunar cycle effect on sleep.

Additional analyses: While we were unable to replicate any of the significant findings reported by Cajochen et al., some of our analyses yielded significant results with respect to lunar class as well (see tables 1 and S1). In the Munich2 sample, the main analysis showed a significant effect of lunar cycle on S4 (see table 1) – which is opposite to the respective finding by Cajochen et al. In the analysis with outlier exclusion (table S1a), this variable did not become significant for the Munich2 sample. WASO turned out to be significantly effected by lunar cycle both in the Munich2 and Basel samples in the analysis with outlier exclusion (table S1a), however, in an opposite direction. While WASO was not significantly affected in the Cajochen et al. sample, it is tempting to interpret the finding of more WASO during full moon compared to new moon in the alternative analysis of the Basel sample as a confirmation of the Cajochen et al. data nevertheless, since it would be consistent with the general idea of a sleep impairing effect of full moon. However, on the background of an opposite finding in the Munich2 sample in the same kind of analysis (less WASO during full moon compared to new moon), it is very unlikely that the effect found in the Basel sample is based on an intrinsically quasilunar physiology, but – if not a chance finding – rather on the lack of control for light in this home-recorded sample.

Follow-up analyses of the interaction effects with lunar class revealed selective significant interactions (see tables S1b-d). Specifically, in the Basel sample, there is a significant interaction between lunar class and gender for total sleep time (TST). By follow up tests, we examined the effects of lunar class on TST separately for male and female participants. Test results showed the effects of lunar class on TST were significant neither in the male sample ($p = .93$) nor in the female sample ($p = .73$). The significant interaction results from a relatively smaller gender difference for TST in lunar class 1 ($t(258) = -3.78, p < .001$) as compared to lunar class 2 ($t(289) = -5.21, p < .001$) and 3 ($t(317) = -5.28, p < .001$). Thus, possible lunar class * gender interaction effects on TST should receive attention in future studies. In addition, there was a significant interaction of lunar class with age for S4 in the Munich2 sample and with age and gender for TST in the Basel sample. Please note that age was entered as a covariate in these analyses, as the age range was rather small in both sample (18 – 35 years and 20 – 43 years, respectively) as compared to the larger age range of the Cajochen et al. study. Thus, an interaction with age as a covariate indicates differences in association strength of the dependent variable with the covariate (age). In the Munich2 sample, the interaction (lunar class * age for S4) was due to a slightly more negative correlation between age and S4 sleep in lunar class 1 ($r(236) = -.13, p = .04$) as compared to lunar class 2 and 3 ($r(258) = -.07, p = .27$ vs. $r(257) = -.05, p = .45$, respectively). In the Basel Sample, the interaction (lunar class*gender*age) was due to a slightly more negative correlation for men in lunar class 1 ($r(83) = -.34, p = .002$) and women in lunar class 3 ($r(219) = -.18, p = .01$) as compared to the other four factorial combinations (women lunar class 1: $r(173) = .12, p = .11$; men lunar class 2: $r(97) = -.003, p = .98$, women lunar class 2: $r(190) = -.11, p = .15$, men lunar class 3 $r(96) = -.02, p = .83$). In sum, our post hoc analyses did not confirm the Cajochen et al. findings: None of the age or gender subgroups were significantly influenced by lunar class.

In general, it is not surprising that multiple testing of three different data sets with several dozens of variables yielded a few significant results. However, none of these confirmed the significant lunar cycle effects found by Cajochen et al.

Acknowledgements

We would like to thank Sue Binkley, Gerhard Klösch, Michael Schredl and Jürgen Zulley for their helpful comments.

Supplemental Data

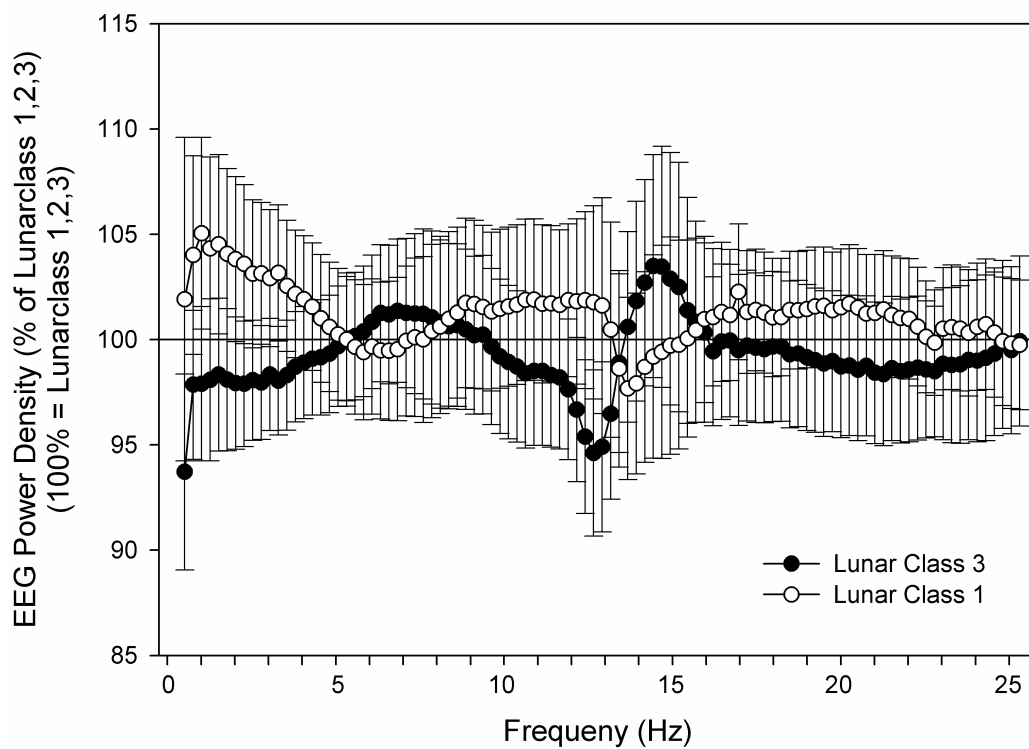
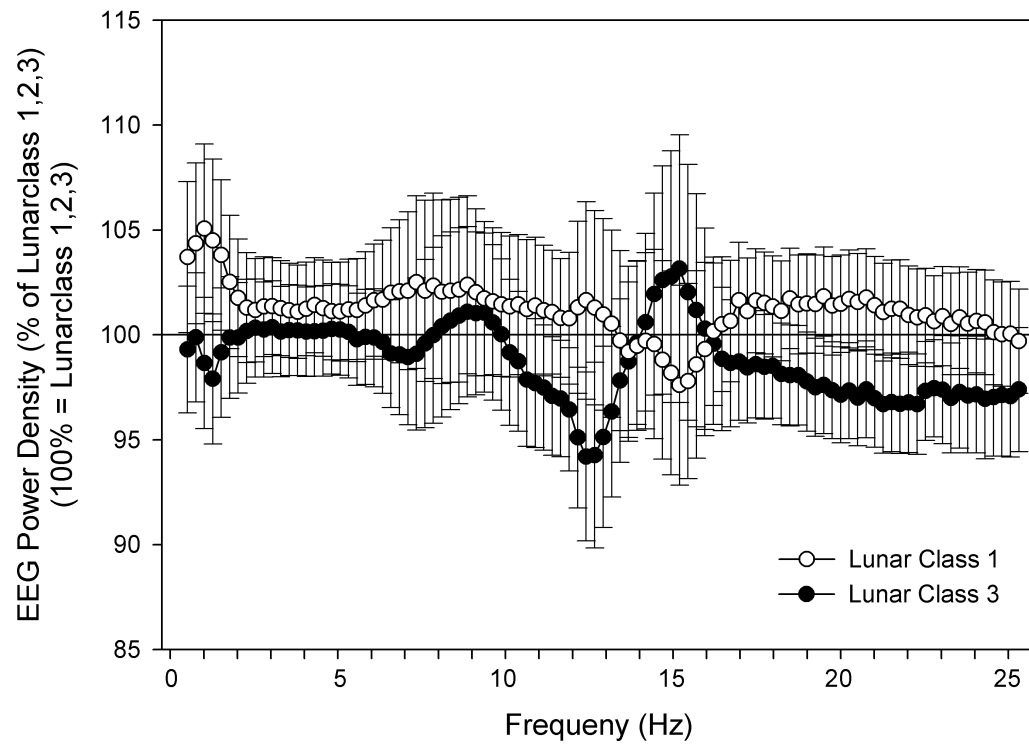


Figure S1. Top: Lunar phase and power density derived from the parietal electrode (Pz). EEG power density between .5 and 25 Hz during NREM sleep for lunar class 1 (around full moon) and 3 (around new moon) for $n = 820$ are indicated. Data of 25 subjects could not be analyzed due to EEG artifacts. Values are percentage of the average of lunar classes 1, 2, and 3. No significant differences emerged for the individual frequency bins. Mean \pm standard error of the mean (SEM) are shown.

Bottom: Lunar phase and power density derived from the occipital electrode (Oz). EEG power density between .5 and 25 Hz during NREM sleep for lunar class 1 (around full moon) and 3 (around new moon) for $n = 763$ are indicated. Data of 82 subjects could not be analyzed due to EEG artifacts. Values are percentage of the average of lunar classes 1, 2, and 3. No significant differences emerged for the individual frequency bins. Mean \pm SEM are shown.

Variable	Lunar Class 1		Lunar Class 2		Lunar Class 3		Sig. for Lunar Class	
	Mean	SEM	Mean	SEM	Mean	SEM		
Munich1 (<i>n</i> = 414)	<i>n</i> = 131		<i>n</i> = 134		<i>n</i> = 149		<i>F</i> _{2,407}	<i>P</i>
TST	428.0	±2.57	430.7	±2.50	433.3	±2.18	.59	.56
WASO	8.9	±.62	8.2	±.60	8.0	±.51	.12	.89
SL2	19.5	±1.41	19.6	±1.50	16.7	±.97	1.74	.18
RL	96.0	±3.46	97.6	±3.24	95.5	±2.54	.10	.90
Stage 1	9.3	±.48	9.1	±.39	8.9	±.41	.25	.78
Stage 2	52.9	±.76	52.7	±.71	52.3	±.63	.12	.89
Stage 3	9.2	±.41	9.2	±.30	10.1	±.34	2.13	.12
Stage 4	7.6	±.62	8.1	±.61	8.3	±.60	.02	.98
SWS	16.8	±.80	17.3	±.66	18.3	±.71	.77	.46
NREM	69.7	±.55	70.0	±.45	70.6	±.51	.56	.57
REM	21.0	±.40	20.9	±.35	20.6	±.36	.43	.65
Munich2 (<i>n</i> = 726)	<i>n</i> = 230		<i>n</i> = 249		<i>n</i> = 247		<i>F</i> _{2,714}	<i>P</i>
TST	442.8	±2.34	441.3	±2.00	439.9	±1.92	1.09	.34
WASO	1.6	±.12	1.6	±.13	1.9	±.14	4.06	.02*
SL2	14.4	±.89	15.0	±.81	15.8	±.84	.23	.79
RL	77.7	±2.26	75.0	±1.87	72.2	±1.64	.04	.96
Stage 1	6.7	±.20	6.9	±.20	7.5	±.25	.11	.90
Stage 2	48.1	±.43	48.4	±.45	49.2	±.46	.58	.56
Stage 3	10.8	±.23	10.8	±.25	10.6	±.23	2.69	.07
Stage 4	10.9	±.42	10.4	±.45	9.5	±.47	2.56	.08
SWS	25.6	±.55	24.5	±.58	23.1	±.30	2.74	.09
NREM	60.8	±.97	61.3	±.91	60.6	±.91	1.75	.18
REM	21.9	±.27	22.0	±.27	21.9	±.27	.26	.77
Basel (<i>n</i> = 845)	<i>n</i> = 253		<i>n</i> = 284		<i>n</i> = 308		<i>F</i> _{2,838}	<i>P</i>
TST	453.6	±4.80	451.5	±4.25	457.0	±4.25	.18	.84
WASO	3.5	±.21	3.2	±.21	2.7	±.15	4.73	.01*
SL2	20.4	±1.64	23.9	±2.10	19.7	±1.56	2.42	.09
RL	87.5	±2.61	87.2	±2.12	84.7	±2.35	.22	.80
Stage 1	3.3	±.12	3.3	±.11	3.3	±.12	.23	.79
Stage 2	49.7	±.49	49.6	±.47	49.5	±.44	.24	.79
Stage 3	11.3	±.31	11.3	±.29	11.3	±.28	.09	.91
Stage 4	16.9	±.42	16.4	±.38	16.4	±.35	.30	.74
SWS	28.2	±.56	27.6	±.53	27.7	±.49	.31	.74
NREM	77.9	±.32	77.2	±.31	77.2	±.30	1.67	.19
REM	18.8	±.28	19.5	±.28	19.6	±.27	2.60	.08

Table S1a. Sleep parameters for lunar classes 1-3 for three different samples (Munich1, Munich2 and Basel) after exclusion of outliers (outlier definition: 3 or more standard deviations from mean in TST, S1, S2, SWS and REM). TST: total sleep time (in minutes); WASO: Wake after sleep onset (in percent of TST); SL2: sleep latency to stage 2 (in min); RL: REM latency (in min), Stage 1-4: sleep stages 1-4 (in percent of TST); SWS: slow wave sleep (sum of stages 3 and 4 in percent of TST); NREM: non-REM sleep (sum of stages 2, 3 and 4, in percent of TST), REM: REM sleep (in percent of TST). Asterisks indicate variables that yielded significance for lunar class ($P \leq .05$). Please note that effects of lunar class on WASO are in the opposite direction for Munich2 and Basel samples.

Variable	Factor	Df	F-value	p-value
TST	Lunar Class	2	.03	.97
	Age	1	93.16	< .001*
	Gender	1	5.72	.02*
	Age*Gender	1	1.86	.17
	Lunar Class *Age	2	.95	.39
	Lunar Class * Gender	2	.65	.52
	Lunar Class * Age * Gender	2	.45	.64
S2_Lat	Lunar Class	2	.20	.82
	Age	1	1.88	.17
	Gender	1	.53	.47
	Age*Gender	1	.31	.58
	Lunar Class *Age	2	.38	.69
	Lunar Class * Gender	2	.17	.85
	Lunar Class * Age * Gender	2	.59	.55
REM_Lat	Lunar Class	2	1.65	.19
	Age	1	3.12	.08
	Gender	1	2.49	.12
	Age*Gender	1	2.86	.09
	Lunar Class *Age	2	.86	.43
	Lunar Class * Gender	2	.09	.92
	Lunar Class * Age * Gender	2	.26	.77
S4	Lunar Class	2	.01	.99
	Age	1	75.36	<.001*
	Gender	1	10.33	.001*
	Age*Gender	1	1.09	.30
	Lunar Class *Age	2	1.05	.35
	Lunar Class * Gender	2	.02	.98
	Lunar Class * Age * Gender	2	1.24	.29

Table S1b, Munich1 sample: Results for the mixed model analysis of the variables for which Cajochen et al. found significant lunar cycle effects. Age (355 young subjects: 18-44 years, mean = 26.23 ± 5.60, 134 females, 221 males; 115 older subjects: 45-74 years, mean = 58.91 ± 7.51, 76 females, 39 males) and gender (210 female subjects, 260 male subjects) were included as fixed factor.

Variable	Factor	Df	F-value	p-value
TST	Lunar Class	2	1.41	.24
	Age	14	29.19	< .001*
	Gender	1	.05	.83
	Age*Gender	2	.11	.74
	Lunar Class *Age	25	1.34	.26
	Lunar Class * Gender	2	.14	.87
	Lunar Class * Age * Gender	4	.03	.97
S2 Lat	Lunar Class	2	.21	.81
	Age	1	7.97	.01*
	Gender	1	.21	.65
	Age*Gender	1	.14	.71
	Lunar Class *Age	2	.28	.76
	Lunar Class * Gender	2	.28	.76
	Lunar Class * Age * Gender	2	.15	.86
REM_Lat	Lunar Class	2	.50	.61
	Age	1	.23	.64
	Gender	1	7.78	.01*
	Age*Gender	1	8.36	.004*
	Lunar Class *Age	2	.65	.53
	Lunar Class * Gender	2	.38	.68
	Lunar Class * Age * Gender	2	.40	.67
S4	Lunar Class	2	3.05	.05*
	Age	1	1.08	.30
	Gender	1	131.82	< .001*
	Age*Gender	1	144.72	< .001*
	Lunar Class *Age	2	3.10	.05*
	Lunar Class * Gender	2	.51	.60
	Lunar Class * Age * Gender	2	.42	.66

Table S1c, Munich2 sample: Results for the mixed model analysis of the variables for which Cajochen et al. found significant lunar cycle effects. As no elderly participants were included in this sample, age was included as covariate and gender as fixed factor.

Variable	Factor	Df	F-value	p-value
TST	Lunar Class	2	.20	.82
	Age	1	5.04	.025*
	Gender	1	.23	.64
	Age*Gender	1	.72	.40
	Lunar Class *Age	2	.21	.81
	Lunar Class * Gender	2	7.32	.001*
	Lunar Class * Age * Gender	2	7.10	.001*
S2 Lat	Lunar Class	2	.15	.86
	Age	1	.29	.59
	Gender	1	.58	.45
	Age*Gender	1	1.68	.20
	Lunar Class *Age	2	.09	.91
	Lunar Class * Gender	2	.89	.41
	Lunar Class * Age * Gender	2	1.24	.29
REM_Lat	Lunar Class	2	2.11	.12
	Age	1	4.25	.04*
	Gender	1	.83	.36
	Age*Gender	1	1.02	.31
	Lunar Class *Age	2	2.31	.10
	Lunar Class * Gender	2	.58	.56
	Lunar Class * Age * Gender	2	.61	.54
S4	Lunar Class	2	.11	.89
	Age	1	72.35	< .001*
	Gender	1	3.28	.07
	Age*Gender	1	1.41	.24
	Lunar Class *Age	2	.14	.87
	Lunar Class * Gender	2	.61	.54
	Lunar Class * Age * Gender	2	.62	.54
Delta EEG	Lunar Class	2	.13	.88
	Age	1	38.33	< .001*
	Gender	1	6.29	.01*
	Age*Gender	1	2.08	.15
	Lunar Class *Age	2	.22	.80
	Lunar Class * Gender	2	.12	.89
	Lunar Class * Age * Gender	2	.35	.70

Table S1d, Basel sample. Results for the mixed model analysis of the variables for which Cajochen et al. found significant lunar cycle effects. As no elderly participants were included in this sample, age was included as covariate and gender as fixed factor.

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